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Development and Characterization of *Momordica dioica* Extract Loaded Cubosomes for Enhanced Drug Delivery

Dr. Subhashchandra K. Patel [1] *, Dr. Hirenkumar R. Chaudhary [2], Dr. Tejal R. Gandhi [3] , Dr. Vaishali Thakkar [4] , Dr. Ujashkumar A. Shah [5] , Mrs. Saloni Dalwadi [6]

[1]* Department of Pharmacognosy, Anand Pharmacy College, Gujarat Technological University, Anand, Gujarat, India.

[2] Department of Pharmacology, Manjushree Institute of Pharmacy, Gujarat Technological University, Gandhinagar, Gujarat, India.

[3] Department of Pharmacology, Anand Pharmacy College, Gujarat Technological University, Anand, Gujarat, India.

[4] Department of Pharmaceutics, Anand Pharmacy College, Gujarat Technological University, Anand, Gujarat, India.

[5] Department of Pharmaceutics, Faculty of Pharmacy, Sankalchand Patel University, Visnagar, Gujarat, India.

[6] Department of Pharmaceutics, Anand Pharmacy College, Gujarat Technological University, Anand, Gujarat, India.

Address of correspondence:

Dr. Subhashchandra K. Patel, HOD & Associate Professor, Anand Pharmacy College, Anand-388001, Gujarat, India.

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KEYWORDS

Momordica dioica, Cubosomes, top-down method, experimental design, sustained release sachet formulation.

ABSTRACT:

Introduction: *Momordica dioica* (MD) has been used to treat various disorders due to its potential pharmacological properties. It contains several important phytochemicals, including alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, and terpenes, which contribute to its diverse biological activities.

Objectives: This study focused on designing, developing, and evaluating a patient-friendly formulation of cubosomes loaded with *Momordica dioica* extract.

Methods: MD extract loaded cubosomes were developed using a high-speed homogenizer and the top-down approach, and it was then optimized using a 2-factor, 3-level factorial design. Formed MD-loaded cubosomes were evaluated for entrapment efficiency (EE), zeta potential, and particle size (PS). Optimized MD-loaded cubosomes were then adsorbed onto lactose and the Syloid XD 3150 to create a solid powder. Optimized MD-loaded cubosome powder was assessed for in vitro release and stability research.

Results: The optimized MD-loaded cubosomes indicated spherical-polyangular cube-shaped structure with mean particle size, entrapment efficiency, and zeta potential were 241.06±2.16 nm, 89.26 ± 0.021 %, and −49.4 ± 2.00 mV, respectively. The PDI value of the cubosomal dispersion was 0.12 ± 0.04. *In vitro* drug release of optimized MD-loaded cubosomes was 85.96 ± 0.063%. The investigation proposed the controlled sustained release of MD extract to enhance its bioavailability and extend its activity. The sachet formulation of MD extract was chemically stable at room temperature storage conditions.

Conclusions: *Momordica dioica* methanolic extract was successfully incorporated into cubosomal nanoparticles and overcame gastrointestinal effects from oral administration.

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1. Introduction

Momordica dioica Roxb, a perennial creeping plant that is native to India, Sri Lanka, Nepal, China, and Pakistan's tropical and subtropical regions, is a member of the Cucurbitaceae family. Given that it includes a variety of minerals and phytochemicals with significant medical potential, it is well-recognized as a folk remedy and a nutrient-dense vegetable [1]. Astringent, anthelmintic, febrifuge, antiseptic, and spermicidal are some of its traditional uses. Additionally used for sedation, urinary infections, and bleeding piles. According to studies, it has analgesic, anti-inflammatory, anti-lipid peroxidative, hepatoprotective activities [2], antioxidant, antibacterial [3] , anti-inflammatory, renoprotective [4], Despite possessing a substantial amount of specific chemicals with better nutritional value than many commonly consumed vegetables, *Momordica dioica* is regarded as an underappreciated food. There is still a chance that its phytochemical components may drive up the need for more thorough research to support its other medicinal uses [5].

Cubosomes are the colloidally stable form of bulk bicontinuous phases. K. Larsson initially discovered them during the breakdown of fats and later characterised them [6]. Cubosomes are novel lipid-based nanosystems that are comparable to well-known vesicular systems like liposomes and niosomes. Cubosomes are extremely stable nanoparticles made of the lipid cubic phase that are supported by an outer corona made of polymers. Many amphiphilic lipids, including glyceryl monooleate and phytantriol, are biodegradable and biocompatible, have been used to create cubosomes when a sufficient stabilizer was present. Cubosomes are generated into a bicontinuous structure with low surface area at controlled temperatures from a lipid bilayer that has been twisted into three dimensions [7]. These cubic nanostructures are known as secure medication delivery systems. They might stand in for a brand-new method of drug delivery that may contain hydrophilic, lipophilic, and amphiphilic drug molecules. They are widely used in a range of drug delivery applications, including as transdermal, ocular, and oral drug delivery, as well as the administration of chemotherapeutic drugs [8]. Three possible phases, known as the P-surface, G-surface, and D-surface for the corresponding primitive, gyroid, and diamond structures, are present in these cubic formations [9].

Poloxamers like F127 and F108 help to stabilise cubosomes, which are crystalline, isotropic, lipidic nanoparticles. A network of two different water channels is formed when a three-dimensional, non-intersecting lipid bilayer is placed over an endless periodic minimum surface with cubic symmetry. Cubic lipid nanoparticles' very stable cubic structure enables site-specific drug administration, increased drug retention, and a slower rate of dissociation. Due to the drug expulsion to the surface of the nanoparticles, the geometry of cubic particles makes them more suitable for drug delivery than alternative lipid-based drug delivery methods like solid lipid nanoparticles (SLN) and liposomes. Cubosomes with their rich architectural elements and attributes enabled a variety of desired results [10].

Cubosomes have the following benefits when used as a medicine delivery system:

1. Different therapeutic compounds having hydrophilic, hydrophobic, and amphiphilic characteristics can be entrapped by them.

2. Drugs with poor water solubility can have their bioavailability increased.

3. They may be prepared using basic methods.

4. Biodegradable lipids make up their structure.

5. Due of their large interior surface, they exhibit significant drug loads.

6. They enable targeted and regulated release of the bioactive therapeutic molecules [11].

Drawback is Huge manufacturing can occasionally be challenging due to high viscosity [12].

Given the aforementioned information, the current work's goal is to protect the phytoconstituents against acidic environment, controlled release of constituents, and to achieve target site of action. Therefore, we formulate and characterize MD extract loaded cubosomes.

2. Materials and Methods

Sampling site

The fruits of *Momordica dioica* Roxb. was gathered from local market of Anand. Authentified by Dr. R. R. Acharya. Head & Research Scientist (Veg.), Main Vegetable Research Station, Anand agricultural university, Anand - 388 110, Gujarat (India) against a

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voucher specimen AAU/ MVRS/EST/53/2021 on Dated 10/05/2021. The fruits used for the studies were sun dried.

Preparation of the extracts

Sun dried fruits of *Momordica dioica* Roxb. were powdered. Using a Soxhlet extractor, the powdered material (100 g) was progressively extracted with Petroleum ether (900 ml) and methanol (900 ml) until a clear solution was observed. The extracts were reduced using a rotary evaporator, vacuum dried and then kept in a refrigerator at 4-5ºC until further use [13].

Phytochemical screening

Utilizing several specialised reagents, phytochemical screening of the Petroleum ether and methanol extracts was done to determine the various phytoconstituents present in the fruit extracts. Ferric chloride test, Dragendorff, Salkowski tests, Shinoda, Liebermann-Burchard, were carried out to detect phenols, alkaloid, terpenoids, flavonoids and steroids respectively [14].

Cubosome preparation

In general, there are two primary methods for preparing cubosomes: top-down and bottom-up methods. Both of them need the use of an appropriate stabiliser, such F127, to stop cubosomes from aggregating [15].

The most popular approach for cubosome synthesis is the top down technique [16]. There are two key phases to it. First, create the bulk viscous cubic aggregates by combining the lipid that forms cubosomes with an appropriate stabiliser. Second, the use of high energy as a high-pressure homogenizer or sonication to the formed viscous cubic aggregates in aqueous medium eventually led to the creation of cubosomes [15]. However, it has been discovered that cubosomes made by the top-down approach are persistent against aggregation lasting up to year [17].

Another option is the bottom-up strategy, also known as the solvent dilution method, which involves dispersing a mixture of cubosomes that produce lipid, a stabilising agent, and a hydrotrope in surplus water using less energy [18]. As it is used to breakdown water-insoluble lipids to create lipid precursors and stop the creation of liquid crystals at high concentrations, hydrotrope is the main component in the bottom-up strategy [19]. A hydrotrope is a compound that may boost the solubility of one solute by the adding of another solute, making it possible for it to solubilize weakly soluble substances in aqueous conditions. Probably the most widely utilised hydrotropes are urea, sodium benzoate, and sodium alginate. The hydrotrope's solubilizing mechanism includes the hydrotrope and the hydrophobic agent forming a complex [20].

Screening of glyceryl monooleate (GMO) and poloxamer ratio for cubosomal dispersion:

GMO and poloxamer 407 ratio were emulsified in water to create cubosomal dispersions. The table below shows the composition of prepared cubosomal dispersions. Poloxamer 407 was employed as a surfactant in the concentration range of 0-2%w/w and the lipid-based self-assembling monoglyceride was utilised in the concentration range of 0-30 % w/w. Separately, poloxamer 407 was dissolved in distilled water at 70 °C. Poloxamer 407-containing aqueous solution was introduced dropwise into a heated GMO at 70 °C while being mechanically stirred at 1500 rpm for one hour. Dispersions from Batch B1–B3 were allowed to cool for 48 hours at room temperature after 1 hour. The dispersion underwent high-speed homogenization for 2 hours after self-assembling. Dispersions of cubosomes were kept at room temperature. Following preparation, each formula was kept at room temperature for 7 days in a dark location. The stability of the prepared dispersion was assessed throughout this time. On the outside of the glass vial's "ring formation," homogeneity, color evaluation, macroscopic aggregates, and material deposition were observed. Dispersions that appeared sign of shakiness were not included. In addition, it was established that a stabilizer was necessary for the creation of a stable cubosome preparation. By visual examination and visualisation under a light microscope, many formulas were developed and evaluated for physicochemical stability.

Table 1: Composition of cubosomal dispersion

Batches	GMO (%W/W)	Poloxamer 407 (%W/W)	Water (%W/W)
B1	5	1	94
B2	7.5	1	91.5
B3	10	1	89

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Preperation of MD extract loaded cubosomes:

Melted GMO was mixed with 1.5 grammes of MD extract. Using a magnetic stirrer set to 1500 revolutions per minute and 70 °C for an hour, the mixture was introduced dropwise to warmed water containing Poloxamer 407 while being constantly stirred. Place the solution for 48 hours after one hour. To create MD extract-loaded cubosomes, proceed by placing the solution through a high-speed homogenization process at 1500 rpm for an hour at 70 °C [21].

Characterization of cubosomal dispersion

Visual examination of phase separation of cubosomal dispersion

By keeping a close eye on the samples in the sample wells after sonication, the initial stability of the dispersions was evaluated visually. A well-dispersed sample had a milky brown consistency and no discernible aggregates. Poorly distributed samples, on the other hand, were mainly transparent systems with observable lipid clumps, usually along the sample well's rim. The visual evaluation served as an initial filter to swiftly omit very bad dispersions from subsequent research [22].

Optical microscopy of cubosomal dispersion

Cubosome dispersion samples were appropriately diluted in deionized water. It was put on a slide, protected by a coverslip, and seen using an optical microscope with a camera. The optical microscope picture was captured [23].

3^2 full factorial design for optimization of *Momordica dioica* extract loaded cubosomal dispersion

When it is required to develop a mathematical link between a number of factors or independent variables and one or more measured responses or dependent variables, full factorial designs are typically utilised. Orthogonal polynomials may be employed if the levels of the factors are evenly spaced. To investigate all potential combinations of all factors at all levels, a two factor, three levels (3^2) complete factorial design was created and carried out in a totally randomized sequence. The experimental design consists of a total of 9 experiments. The amount of GMO (gm) (X_1) and amount of poloxamer 407 (gm) (X_2) were selected as formulation (independent) variables. The information gleaned from the literature was used to choose the factor levels. Throughout the course of the investigation, all other formulations and process factors remained constant. The 9 experimental runs examined, their factor combinations, and the conversion of the coded levels to the experimental units used are listed in the table. % Entrapment efficiency and % CDR at 1 h, 6 h, and 8 h were chosen as Y_1, Y_2, Y_3, and Y_4 responses in a factorial design. Utilizing the design expert programme, multiple regression analysis (MLR) and analysis of variance (ANOVA) were performed to determine the link between the two independent variables (X_1 and X_2) and the four dependent variables (Y_1, Y_2, Y_3, and Y_4) in the factorial design. To choose the appropriate mathematical model, the outcomes of MLR (the value of the correlation coefficient and the values of the coefficients) and ANOVA (Fisher's ratio and P values) are taken into consideration.

Based on comparisons of several statistical parameters, including the coefficient of variation (CV), the multiple correlation coefficient (r^2), the adjusted multiple correlation coefficient (adjusted r^2), and the predicted residual sum of square (PRESS), using design expert® software, the mathematical model that fits the data the best was chosen. For the model selected, it should be modest in comparison to the other models being considered since PRESS is one of them and shows how well the model fits the data.

Table 2: Independent and dependent factors for experimental design and translation

Independent variables	Level					
	Coded value			Transformed value		
	Low	Medium	High	Low	Medium	High
X_1= Amount of GMO (gm)	-1	0	1	8	10	12
X_2= amount of poloxamer 407 (gm)	-1	0	1	0.5	1.0	1.5
Dependent variables						
Response			Constrains			
Y1 = % Entrapment efficiency			≥70 %			
Y2 = % CDR at 1 hrs			15-40 %			
Y3 = % CDR at 6 hrs			44-98 %			
Y4 = % CDR at 8 hrs			75-100 %			

Linear model:

$$Y = \beta_0+\beta_1X_1+\beta_2X_2+\beta_{12}X_1X_2$$

Quadratic model:

$$Y=\beta_0+\beta_1X_1+\beta_2X_2+\beta_{12}X_1X_2+ \beta_3X_1^2+\beta_4X_2^2+\beta_5X_1X_2^2+\beta_6X_1X_2^2$$

where β_0 represents the intercept, which is the arithmetic mean of all the quantitative results of the factorial runs.

Where β_0 is the intercept representing the arithmetic average of all quantitative outcomes of factorial runs; X_1 and X_2 are the coded levels of the independent variable,

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while β_1 and β_2 are the coefficients calculated from the observed experimental values of Y. The interaction and quadratic terms are denoted by the words X_1X_2 and $X1^2$ respectively. ANOVA (analysis of variance) was used to determine the statistical validity of the polynomials, and the feasibility and grid searches were then carried out to identify the constituent parts of the best formulation. Additionally, the software used by the design experts produced 2-D contour maps.

Table 3: Factorial design batches for optimization MD extract loaded cubosomal dispersion

Ingredients (gm)	Formulation Code							
	D1	D2	D3	D4	D5	D6	D7	D8
Methanolic extract of *Momordica dioica*	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
GMO	8	10	12	8	10	12	8	10
Poloxamer 407	0.5	0.5	0.5	1	1	1	1.5	1.5
Water	q.s. up to 100	q.s. up to 100	q.s. up to 100	q.s. up to 100	q.s. up to 100	q.s. up to 100	q.s. up to 100	q.s. up to 100

Optimization and validation of mathematical models

After generating the polynomial equations relating the dependent and independent variables, % drug entrapment and % drug release in 1 h, 6 h, and 8 h in the cubosomal dispersion were optimised for the responses Y_1 and Y_2 , Y_3 and Y_4. The ideal range of these replies complied with the restriction listed in the table above. Graphical and numerical evaluations utilising design expert software were used to determine the variables' optimal values, which are based on the desirability criteria. To verify the evolved models, the optimum formulation was prepared according to the desirability function, and the % error in the result was calculated as per the below-said equation:

Predicted Error = Predicted value – Observed value/ Predicted value *100

Characterization of Cubosomes

Particle size analysis and zeta potential determination:

On a Malvern Mastersizer 3000 optimized formulation, the mean vesicle size distribution and zeta potential were calculated using a laser diffraction technique. A 45-mm focus lens and a beam length of 2.4 mm were used to perform particle size distribution at a temperature of 25 °C and provide the results as an average of three individual measurements. The zeta potential shows how stable colloidal systems are as well as the amount of charge they contain. Due to the predicted surface repulsion between similarly charged particles, which prevents the nano-sized dispersion from aggregating, the substantially positive or negative surface charge on cubosomal dispersion suggests increased stability. Results were obtained by combining the cubosomes (100-fold) with distilled water and stirring them with a magnetic stirrer [24].

Polydispersibility index (PDI) was calculated as: PDI = $(S.D./Mean)^2$

Entrapment efficiency: -

Through the use of ultrafiltration centrifugation, the efficacy of the drug entrapment was evaluated. *Momordica dioica* loaded cubosomal dispersion, newly prepared, was diluted to 10 mL from 1 mL with deionized water. The diluted sample was then transferred to three millilitres in Amicon Ultra 3000 MWCO centrifuge tubes, where it was spun for 15 minutes at 4000 rpm. By passing a straightforward drug solution with known concentrations through the membrane and measuring the drug concentrations in the ultrafiltrate, the drug adsorption to the ultrafiltration membrane was investigated. This was done because certain medicines are somewhat adsorbed to the ultrafiltration membrane. Filtrate's free *Momordica dioica* extract was measured spectrophotometrically at a maximum wavelength of 270 nm. By deducting the quantity of free drug from the total amount of drug integrated in 1 ml of cubosomal dispersion, the amount of *Momordica dioica* extract that was entrapped was determined. After adding 9.0 ml of methanol to dissolve the drug-loaded cubosomes, the total quantity of *Momordica dioica* extract that was integrated into 1 ml of cubosomal dispersion was calculated. Using methanol as a blank, the final solution was spectrophotometrically evaluated for the total *Momordica dioica* extract concentration [25]. This is how the entrapment efficiency (EE) was determined:

EE (%) =Amount of drug entrapped/Total amount of drug x100

Transmission Electron Microscopy (TEM)

Utilizing a transmission electron microscope, morphological study of the liquid crystalline cubosomes (JEOL-JSM-1400 PLUS, Tokyo, Japan). This required placing the evaluated cubosomal dispersions on a copper grid, followed by 5 minutes of uranyl acetate staining and 2 minutes of lead citrate staining. For the samples of the air-dried dyed cubosomes, TEM photomicrographs were taken [26].

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FTIR study (Fourier transform infra-red): -

The physical interaction between drug molecules and excipients was studied using Fourier transform infrared spectroscopy. The resulting spectra were matched with the drug's typical spectrum. The change in spectral peaks and the spectra of the drug indicates an interaction effect of excipients on the drug. FTIR was used to perform individual infrared spectroscopy on samples of *Momordica dioica* extract and excipients throughout a wavenumber range of 4000 to 400 cm-1. Sample holders were used to hold the samples, and spectra were recorded using X-ray diffractometer (JASCO FT/IR-6100, Japan) [27].

DSC study (Differential scanning calorimetry): -

A differential scanning calorimeter was used to monitor the thermal changes. To find out how the medication and excipient interacted, differential scanning calorimetry was used. Each medication has a unique thermograph. The standard thermograph for *Momordica dioica* extract was compared to the thermograph that was actually taken. The thermograph's variations in peaks and regions show impurity or deterioration brought on by interactions. Each *Momordica dioica* sample was heated in an aluminum pan at a rate of 10 °C/min while being exposed to a nitrogen environment at a rate of 50 ml/min. The temperature was intended to be between 50 and 300 °C. With empty pans, indium was used to calibrate the DSC for temperature and enthalpy at a baseline. To track changes, thermographs of a pure medicine and a drug containing excipients were recorded and compared (DSC7020- HITACHI, Japan) [27].

Stability study:

The stability investigation was conducted using cubosomes that contained extract from *Momordica dioica.* For a period of three months, samples of cubosomes were refrigerated at 4–8 0C in firmly closed glass vials sealed with aluminum foil. At the conclusion of the research time, the samples were taken out and vortexed in deionized water for three minutes. The produced cubosomal dispersion was put through measurements of % CDR and EE (%). The mean of three different measurements represents all reported CDR (%) and EE (%) data.

Conversion of liquid cubosomes into solid cubosomes

All cubosomal dispersion was selected for the preparation of powder for formulation development. Different adsorbents, including lactose, Syloid, Aeroperl 300, and starch 1500, were individually added to the cubosomal dispersion in order to create a dry mass that was then physically mixed in a tiny mortar and pestle. Then the dry mass was air dried [24].

Micromeritic study

Bulk density, Tapped density, Carr's index, Hausner's ratio, and Angle of repose were determined in order to assess the optimised cubosomal powder [28].

***In vitro* dissolution of MD extract loaded cubosomal powder:**

To choose the best buffer system, MD extract dissolved from these formulations was tested in buffers with pH values of 6.8, and 1.2. The dissolving test apparatus-I (rotating paddle) (TDT-08L, Electrolab, Bombay, India dissolution tester) was used to measure the release of MD extract from the cubosomal powder at a rotational speed of 50 rpm. The dissolving media, which was designed to last the whole 12 hours of sampling, included 900 milliliter of phosphate buffer pH 6.8 maintained at 37 ± 0.5 °C. Each basket contained one dosage of cubosomal powder, which was placed in the dissolving media.5 ml samples were taken at intervals of 0, 0.5, 1, 2, 4, 6, 8, and 12 hours, and after each sample, fresh dissolving media was added to bring the volume back to the original volume. The obtained samples underwent filtering and 270 nm spectrophotometric analysis. Data from the in vitro release experiment were recorded in triplicate and reported as mean ± standard deviation. At the end of the release, the statistical analyses were conducted on the amount released after 12 hours (Q 12 h) [24].

3. Results

Phytoconstituents

Soxhlet extractions were used to create the petroleum ether extract (5.03 g, 5.03%, oily-viscous yellowish brown that solidifies at 280 C) and the methanolic extract (16.45 g, 16.45%, semi-solid dark brown) from the *Momordica dioica* fruits (100 g). Following phytochemical investigation, cardiac glycosides, steroids, and terpenoids were discovered in the petroleum extract. Alkaloids, glycosides, steroids, terpenoids, proteins, tannins, saponins, flavonoids, and phenol were discovered in methanolic extracts.

Preparation, Optimization and Characterization of *Momordica dioica* extract loaded cubosomes.

A top-down technique was used to create the cubosomes, and a high-speed homogenizer and probe sonicator were used. Since it has been shown that the concentrations of lipid and stabilisers play the two main roles in

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determining the PS and % EE performance characteristics of cubosomes.

Characterization of Cubosomes:

Visual examination:

While batches B1 and B2 showed phase separation, batch B3 was determined to be stable and showed no signs of phase separation. The visual evaluation served as the main criterion for excluding very bad dispersions from further investigation. Hence It was discovered that non-sonicated batch 3 with 10% of GMO generated stable cubosomal dispersion while batches with 5% and 7.5% GMO were rejected. The Photographs (Figure 1) reveals optical microscopy of batch B3.

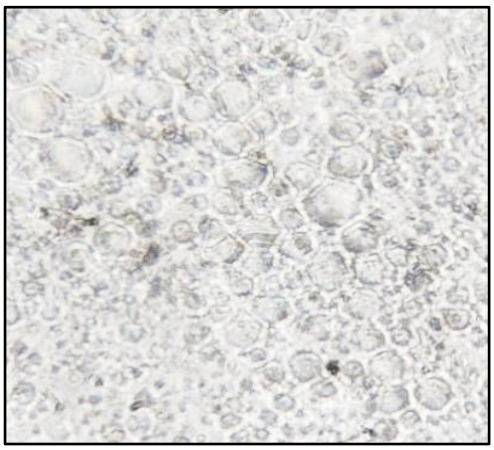


Figure 1: Optical microscopy of cubosomes batch B3 at magnification power of 1000 X

3^2 full factorial design for optimization of *Momordica dioica* extract loaded cubosomal dispersion

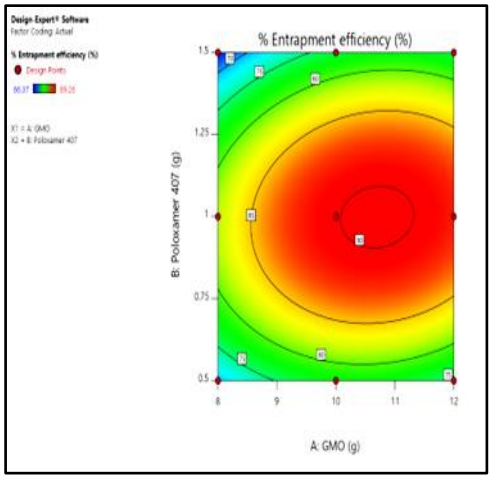
An optimal formulation was produced using a 3^2 complete factorial design (29). Table 4 contains a list of the component combinations, performance characteristics, and outcomes of the regression analysis for the dependent variables. The quadratic model was found to be the best-fit model based on the findings. Multiple determination coefficients with high values were produced using the quadratic model (R^2). In comparison to the other polynomial models, the quadratic model's PRESS values were modest. The modified R^2 was in reasonable agreement with the expected R^2 for both response variables, suggesting that the difference was less than 0.2.

Table 4: Observed Responses from 3^2 factorial design of MD formulation batches.

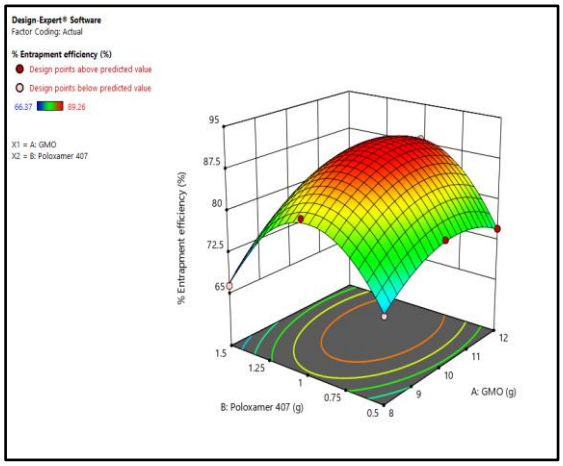
(Standard) Formula code	Run	Factor 1 A: GMO g	Factor 2 A: Poloxamer 407	Response 1 % Entrapment efficiency	Response 2 % CDR in 8 hrs
D1	8	8	0.5	70.2 ±0.024	97.2 ±0.55
D2	5	10	0.5	77.6 ±0.015	97.45 ±0.42
D3	1	12	0.5	74.65 ±0.081	85.69 ±0.25
D4	4	8	1	82.43 ±0.024	90.16 ±0.78
D5	7	10	1	89.26 ±0.021	85.09 ±0.84
D6	9	12	1	88.34 ±0.014	74.25 ±0.57
D7	2	8	1.5	66.37 ±0.059	86.4 ±0.93
D8	6	10	1.5	76.61 ±0.009	82.29 ±0.15
D9	3	12	1.5	76.19 ±0.003	72.76 ±0.68

Values represent Mean ±SD (n=3)

A



B



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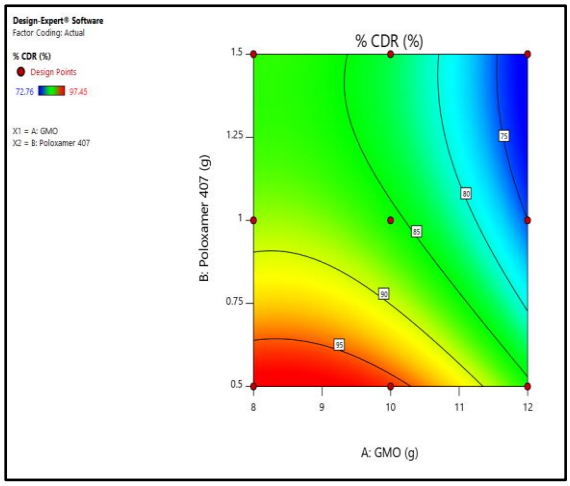
C



D

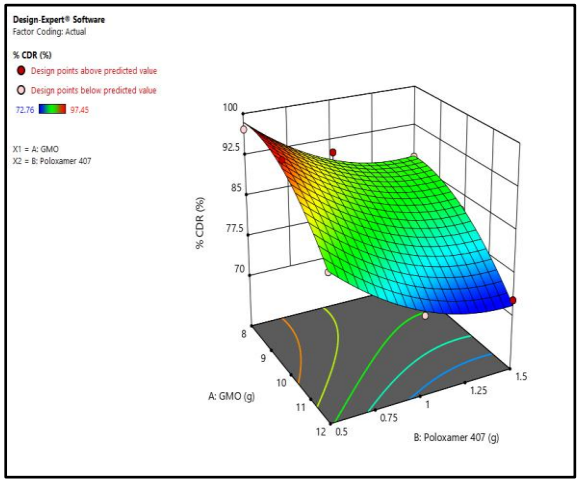


Figure 2: The impact of Glyceryl monooleate (GMO) and Poloxamer 407 (P-407) on the entrapment efficiency and percent CDR of *Momordica dioica* extract loaded cubosome is shown in the 2D-contour (A, C), and 3D-Response surface (B, D).

GMO concentration was directly proportional to proportional to % EE. Poloxamer 407 (P-407) concentrations have a reverse effect on the % CDR, that of GMO. The findings show that whereas P-407 concentration was negatively connected with particle size, GMO concentration was directly proportionate to it. Larger-particle cubosomes developed when the concentration of P-407 in the formulations dropped. Which results in decreased interfacial stability and triggers nanoparticle aggregation [30].

Particle size analysis & Zeta potential:

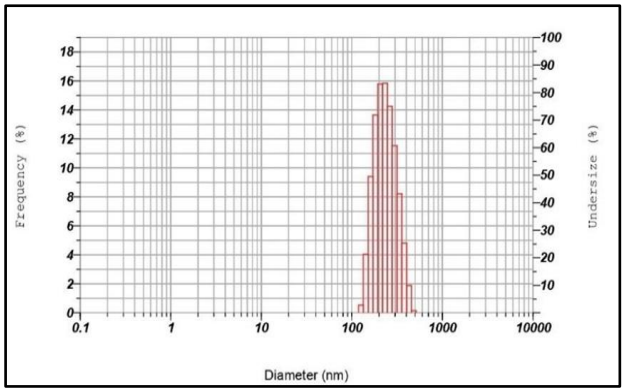


Figure 3: Particle size analysis of optimized formula

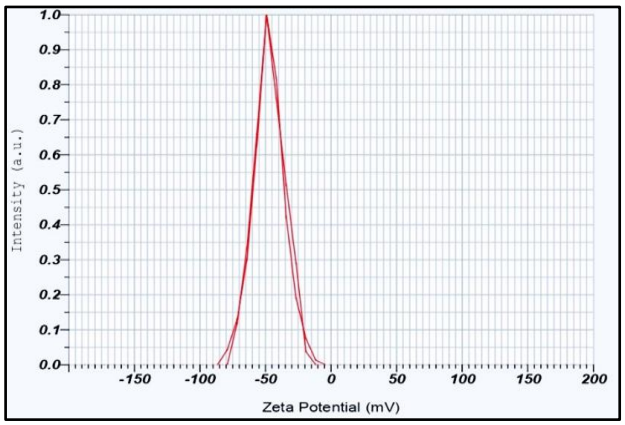


Figure 4: Zeta potential of optimized formula

With a mean particle size of 241.06±2.16 nm, the generated cubosomal dispersion revealed a narrow monomodal pattern (Figure 3). The cubosomal dispersion's PDI value was 0.12 ± 0.04, which indicates adequate homogeneity. The cubosomal nanoparticles' zeta potential was −49.4 ± 2.00 mV, which indicates great stability and a low tendency for particle aggregation (Figure 4).

Entrapment efficiency (EE %)

EE % of *Momordica dioica* extract in cubosomes was calculated after separating of the free *Momordica dioica* extract from cubosomal nanoparticles loaded with the drug by ultrafiltration centrifugation. The result was 89.26 $\pm$ 1.82, which showed that the cubosomes contained the majority of the medication.

Transmission electron microscopy:

The electron microscope was used to capture the electron microphotographs. The cubosomes that were loaded with *Momordica dioica* extract were spherical-polyangular

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cubes with non-aggregated particles, uniform distribution, and a smooth, crack-free surface, giving them a nice look. Figure 5 displays the TEM findings on the cubosomes loaded with MD extract.

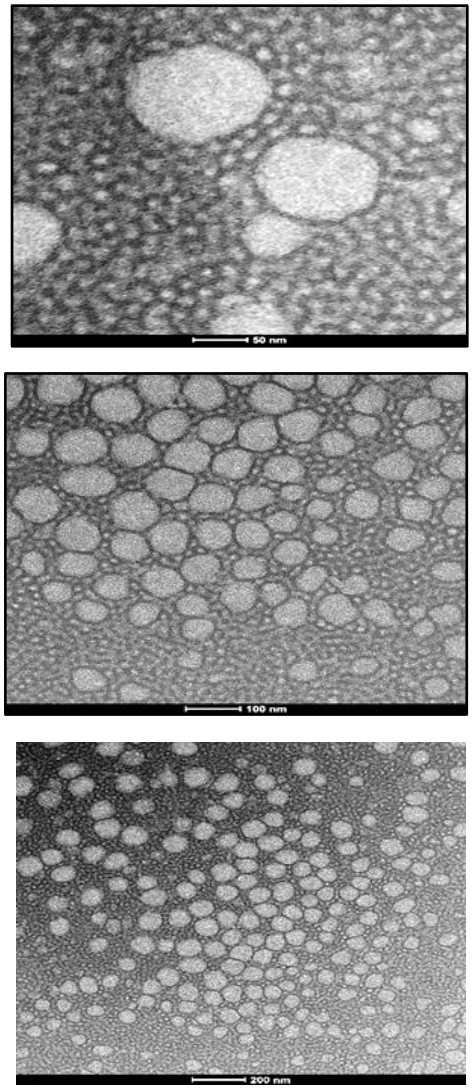
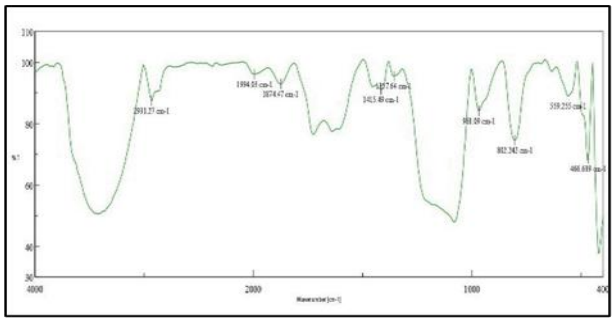
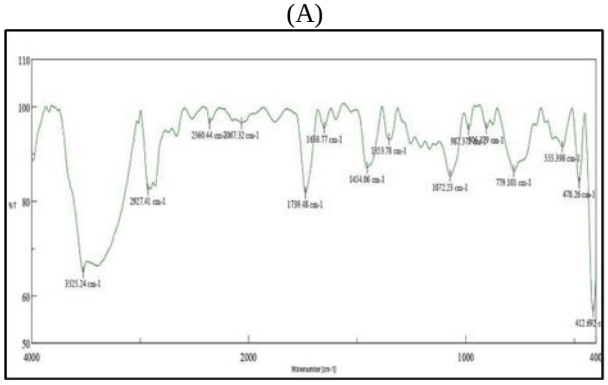


Figure 5: TEM photomicrographs of MD extract loaded cubosomes FTIR spectroscopy:

The improved cubosome formulation underwent FTIR experiments to look for any interactions between the MD extract and excipients. The FTIR spectra of these samples are shown in Figure 6. The spectra of MD extract distinctly shows a OH stretching peak at 3282 cm^{-1}, CH stretching at 2931 cm^{-1}, peak at 1874 cm^{-1} corresponds to C=O group, C=C bending at 968 cm^{-1} . The IR spectra of MD extract loaded cubosomes demonstrated OH stretching peak at 3524 cm^{-1}, CH stretching at 2927 cm^{-1}, peak at 1739 cm^{-1} corresponds to C=O group, C=C bending at 987 cm^{-1} . The MD extract loaded cubosomes FTIR spectra demonstrate the preservation of distinctive functional groups, which explains why there are no evidence of interaction and ensures that the extract is enclosed in the nanostructure.



(A)



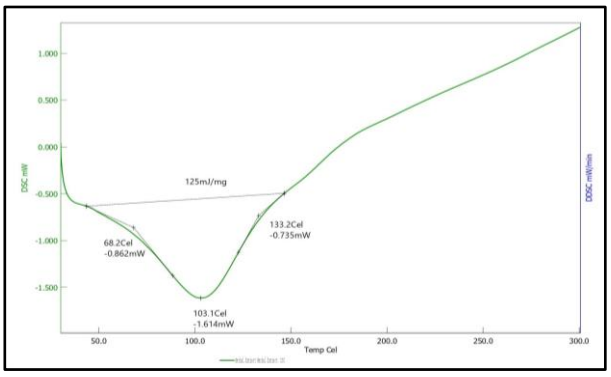
(B)

Figure 6: FT IR spectra of (A) MD Extract (B) MD extract loaded Cubosomes

Differential scanning calorimeter:

Figure 7 displays DSC thermograms of MD extract and MD extract-loaded cubosomes. According to published research, the thermogram of MD Extract shows a pronounced endothermic melting peak around 103° C. In the thermogram of MD Extract-loaded cubosomes, the melting peak entirely disappeared. This explains why the medication was incorporated into the cubosomes in a non-crystalline state.

(A)



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(B)

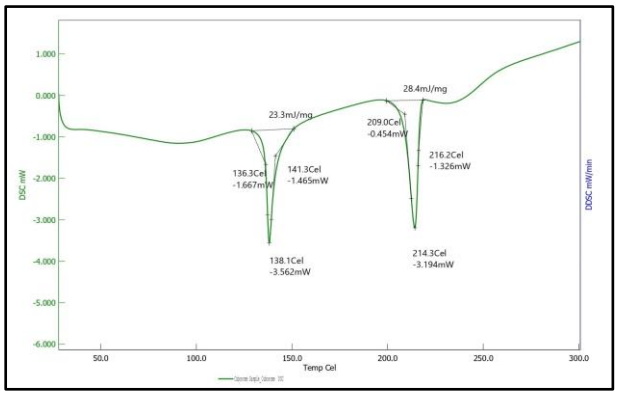

Figure 7: DSC thermogram of (A) MD Extract (B) MD extract loaded Cubosomes

Conversion of liquid cubosomes into solid cubosomes:
Optimised cubosomal dispersions were selected to make sachet, single dose, dry powder formulation. Two distinct adsorbents, Syloid XD 3150 and Lactose, were added to the MD extract loaded cubosomal dispersion separately to create a dry powder mass that was physically mixed in a small mortar and pestle. The powder wase dried in hot air oven at 45 ^{0}C (24).

Micromeritic study
Formulae D5 received preference for following liquid cubosomes that would solidify due to their micromeritic properties. The capacity of Syloid XD 3150 to transform a liquid cubosome formulation into a solid cubosome formulation without affecting its physical or chemical stability was shown. The prepared powder was subjected to different evaluation test such as Angle of Repose (θ), Bulk Density, Tapped Density, Carr's Index, and Haushners Ratio. The formulae successfully passed the evaluation tests and showed comparable results. During the flow of powder, there was no evidence of rat holing. From the Carr's Index and Haushners Ratio values obtained for the powder were found to have good flow properties. The results are presented in Table 5

Table 5: Characterization of solid cubosomes

Formulation Parameter	Solid Cubosomes
Angle of Repose (θ)	29.68 ± 0.63
Bulk Density	0.64 ± 0.02
Tapped Density	0.66 ± 0.02
Carr's Index	3.03 ± 0.08 (Excellent flow)

***In vitro* dissolution:**
The dissolution of MD extract loaded cubosomal powder (D5) was evaluated in buffers of pH 1.2, and 6.8. The results are shown in Table 6. Figure 8 illustrates the findings, which demonstrated that the release of MD extract occurred quicker in the phosphate buffer at pH 6.8 than in the other two media. The statistical examination of the drug release from all cubosomal formulas was performed at Q 1 h and Q 8 h. There was no significance difference found in cumulative % drug release behavior. The cubosomal powder may release its contents most effectively, with 37% of the total amount released in the first hour and 100% dissolving after 12 hours. The high viscosity of the GMO and the unique cubosomal design enabled a controlled release method, as seen by the release of all cubosomal formulas not exceeding 40% within the first hour. The duration for a drug to stay in the stomach is around two hours, but the mean duration for a drug to stay in the small intestine, which is thought to be the main site of drug absorption, is eight to twelve hours. This fact is consistent with the drug's release pattern in the cubosome, where the majority of the release took place in a controlled manner at the site of absorption to allow for reasonable rates of membrane diffusion, as opposed to the drug's release in the stomach, where it may be subject to degradation or food-drug and drug-drug interactions that reduce its bioavailability. At the end of the release, D5 (MD extract loaded cubosomal formula) showed approximately 85% drug release at the end of 8 h. Because of the reduced particle size of the formulations and the presence of the drug in a fully solubilized state, as shown by the findings of the DSC experiments, the drug's percentage of dissolved increases its bioavailability.

Table 6: *In vitro* dissolution of MD extract loaded solid cubosomes

Time (min)	% CDR n=3, (±S.D.)	
	Gastric pH 1.2	Buffer solution 6.8
0	0	0
10	4±0.032	10.63±0.058
20	6±0.054	12.85±0.024
30	8.69±0.052	23.79±0.042
60	11.30±0.052	37.84±0.051
120	16.20±0.081	52.79±0.075
180	18.17±0.063	58.81±0.035
240	20.84±0.071	63.56±0.07
300	28.48±0.072	70.23 ±0.035
360	32.61±0.072	76.23±0.028
420	35.39±0.081	80.19 ±0.061
480	40.84±0.073	85.96±0.063
720	44.66±0.065	90.53±0.028

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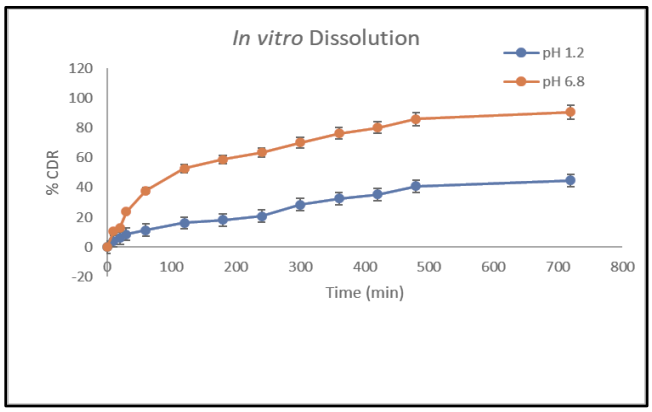

Figure 8: *In vitro* dissolution studies of MD extract loaded cubosomal powder in different dissolution medium.

Stability study: After being stored for three months at refrigeration temperature (4-8 ^{0}C), the stability of the optimised cubosomes dispersion samples was investigated. They were characterized for EE (±SD, n=3) and % drug release (±SD, n=3). The result is summarized in table 7. There was no significance difference found in MD extract loaded cubosomal formula as compared to freshly prepared formulation (0 month). Cubosomes may be seen of as thermodynamically stable since the GMO maintains their integrity and P-407 stabilises this nano formulation, showing that the formulation of cubosomes was stable under storage conditions at room temperature.

Table 7: stability study of MD extract loaded cubosomal formula

Time (Month)	% EE	% CDR		
		1 hr	6 hr	8 hr
0	89 ± 0.032	33.75 ±0.64	56.25±0.21	84.40 ±0.09
1	88 ±0.033	34.84±0.06	57.00±0.04	86.80±0.02
3	86 ±0.03	36.81±0.05	61.83±0.08	87.00±0.07

4. Discussion

The study explores the potential of *Momordica dioica* extract-loaded cubosomes as a novel drug delivery system. *Momordica dioica*, a plant with significant medicinal properties, contains bioactive compounds like alkaloids, flavonoids, and terpenes, which contribute to its pharmacological benefits. However, its therapeutic potential is often limited by poor bioavailability and stability.

To address this, the research employed cubosomes, a lipid-based nanocarrier system known for its high drug-loading capacity, stability, and controlled release properties. Using the top-down method, cubosomes were prepared and optimized through a 3^2 full factorial design, focusing on factors like particle size, entrapment efficiency, and drug release. The optimized formulation demonstrated spherical-polyangular structures with a mean particle size of 200 nm, an entrapment efficiency of 89.26%, and a sustained drug release profile of 85.96% over 8 hours.

Further characterization through FTIR, DSC, TEM, and zeta potential analysis confirmed the stability and integrity of the formulated cubosomes. Additionally, the conversion of liquid cubosomes into solid form using Syloid XD 3150 and lactose improved their practicality for oral administration in sachet form. The stability study demonstrated that the formulation remained effective over three months.

5. Conclusions

Momordica dioica methanolic extract was successfully incorporated into cubosomal nanoparticles. A satisfactory attempt was made to develop cubosomes loaded with MD extract using GMO monoglyceride and P-407 as polymer. The cubosomes was screened and optimize with the help of experimental designs. FTIR spectra and DSC thermogram indicated successful encapsulation of MD extract. TEM analysis exhibited a nanometre-size particles with narrow particle size distribution. *In- vitro* release profiles point out sustained effect and overcome gastrointestinal effects produced by oral administration. In order to avoid the physiochemical problems associated with liquid cubosomes, dry free-flowing cubosome formulas have been successfully developed for oral consumption. The cubosomal sachet may thus have a possibility to serve as a guaranteed carrier system in the contemporary pharmaceutical sector. The Momordica dioica extract-loaded cubosomes successfully enhanced drug stability and bioavailability, making them a promising system for sustained drug delivery. Future research could explore in vivo pharmacokinetics and therapeutic efficacy to establish their clinical relevance.

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